PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED

WO 9603437A1

(51) International Patent Classification 6 :
C07K 14/635, A61K 38/29

A1

(43) International Publication Date: 8 February 1996 (08.02.96)

(21) International Application Number: PCT/EP95/02993

(22) International Filing Date: 27 July 1995 (27.07.95)

(30) Priority Data:

9415254.3	28 July 1994 (28.07.94)	GB
9415255.0	28 July 1994 (28.07.94)	GB

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PTH OR PTHrP ANTAGONISTS

(57) Abstract

PTH or PTHrP compounds having potent antagonistic activity at the PTH/PTHrP receptor in which at least one of the amino acid residues naturally occurring in positions 2 and 10 is replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain, and optionally at least one of the amino acid residues naturally occurring in positions 3 and 6 is further replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain, having pharmacological activity, e.g. prevention or treatment of conditions which are associated with increased plasma calcium caused by excessive release of PTH or PTHrP.

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PTH or PTHrP antagonists

The present invention relates to parathyroid hormone (PTH) and parathyroid hormone related peptide (PTHrP) compounds, a process for their production, pharmaceutical preparations comprising them and their use as a pharmaceutical.

More particularly the present invention provides a PTH or PTHrP compound having potent antagonistic activity at the PTH/PTHrP receptor in which at least one of the amino acid residues naturally occurring in positions 2 and 10 is replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain, and optionally at least one of the amino acid residues naturally occurring in positions 3 and 6 is further replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain. The compounds are hereinafter referred to as compounds of the invention.

In a particular embodiment of the invention there is provided a PTH or PTHrP compound in which the amino acid residue naturally occurring in position 10 is replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain, and optionally at least one of the amino acid residues naturally occurring in positions 3 and 6 is further replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain.

The term "PTHrP" refers to any naturally occurring form of PTHrP, e.g. human, bovine, chicken, rat or mouse PTHrP. For consistency and as is conventional, in the following description, the same numbering system will be applied to the amino acid residues of the PTHrP sequence regardless of whether any α-amino acid residue of the PTHrP sequence is replaced or omitted according to the invention.

The term "PTH" refers to any naturally occurring form of PTH, e.g. human, bovine, chicken, rat or mouse PTH. For consistency and as is conventional, in the following description, the same numbering system will be applied to the amino acid residues of the PTH

sequence regardless of whether any α-amino acid residue of the PTH sequence is replaced or omitted according to the invention.

By "PTHrP compound" or "PTH compound" is meant a peptide comprising an amino acid sequence based on a N-terminal fragment of PTHrP or PTH respectively, preferably based on a PTHrP or PTH fragment starting with any one of the residues 1-7 and terminating with any one of the residues from 27 to 38 e.g. a N-terminal fragment of PTHrP or PTH comprising up to 31, 34, 35, 36, 37 or 38 amino acid residues. The terms "PTHrP or PTH" will thus be understood as embracing peptides wherein one or more amino acid residues of the said N-terminal fragment is omitted, preferably at the N-terminus. The terms are also to be understood as embracing peptides wherein one or more amino acid residues of the naturally occurring sequence is replaced by one or more other amino acid residues (natural or non natural) in addition to the substitution in position 2 and/or 10 and optionally in 3 and/or 6 according to the invention. The 1-38, 1-34 and 1-31 N-terminal fragments of human PTHrP have the sequences as indicated in SEQ ID No:1, 2 or 3, respectively.

The 1-38, 1-34 and 1-31 N-terminal fragments of human PTH have the sequences as indicated in SEQ ID No:4, 5 or 6, respectively.

The N-terminus of the PTHrP or PTH compounds may be a free or a protected amino group, bearing e.g. a N-protecting group as disclosed in "Protective Groups in Organic Synthesis", T.W. Greene, J. Wiley & Sons NY (1981), 219-287, the contents of which being herein incorporated by reference, preferably protected by R*-CO-, R*-O-CO-, R*-O-CH_2-CO- or R*-SO_2, or an amino group bearing a radical R''', R'''-NH-CO- or R'''-NH-CS- such as defined hereunder.

The C-terminus of the compounds of the invention may be COOH, $CONH_2$ or a mono- or disubstituted amide, e.g. -$CONR_cR_d$ wherein one of R_c and R_d is H and the other is an aliphatic residue, e.g. C_{1-6}alkyl, or both are an aliphatic residue, or R_c and R_d together with the nitrogen atom to which they are attached form a heterocyclic residue, e.g. pyrrolidinyl or piperidinyl.
PTHrP or PTH compounds in accordance with the invention have potent antagonistic activity at the PTH/PTHrP receptor e.g. bind to the PTH/PTHrP receptor, have an intrinsic activity (i.a) for activation of the PTH/PTHrP receptor in a functional bioassay significantly <1, e.g. an i.a of at most 0.3, and antagonize PTHrP or PTH or a

fragment thereof e.g. PTHrP(1-34) or PTH(1-34) in a functional bioassay with a pA2 value of at least 6.5. Preferably compounds in accordance with the invention have an i.a of 0.03 or lower or even not detectable in some of the assays. Example of a functional bioassay is the osteosarcoma-based adenylate cyclase assay employed conventionally in the art. This assay provides an in vitro determination of the extent to which the compound to be tested stimulates adenylate cyclase activity or antagonizes the effect of PTHrP or PTH or a fragment thereof in rat osteosarcoma cells of the UMR lineage, e.g. UMR-106-06 according to the method of Marcus and Aurbach in Endocrinology, 85, 801-810 (1969) as disclosed hereinafter.

By amino acid is meant a naturally occurring or commercially available or non natural amino acid or an optical isomer thereof. A non natural amino acid is an amino acid which is not incorporated into a protein under mRNA direction, e.g. β-Nal, a fluoro-α-amino acid such as fluoroalanine, cyclohexylalanine or trimethylsilyl-alanine.
"Natural amino acids" refer to those well known in the art. They are listed and standard abbreviations are provided in the U.S.P.T.O. publication, Trademark Official Gazette, published May 15, 1990, p. 33 at 46. These amino acids and abbreviations are specifically incorporated herein by reference.
The natural amino acids are shown below:

A	Ala	alanine
D	Asp	aspartic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
Q	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine
V	Val	valine

W	Trp	tryptophane
Y	Tyr	tyrosine

By amino acid residue bearing an aromatic or heteroaromatic group on its side chain is meant an amino acid residue wherein the side chain is e.g. optionally ring-substituted phenyl-methyl, 1- or 2-naphthyl-methyl, 1- or 2-naphthyl-ethyl, 3- or 4-pyridyl-methyl, 3-indolyl-methyl or 3-indazolyl-methyl; preferably it is an amino acid residue of formula -NH-CHR'-CO- as defined below.

According to a preferred embodiment of the invention, there is provided a compound of formula I

$$R\text{-}[(X^2)_p,(X^3)_q,(X^6)_s,R^{10}]\text{-}D\text{-}(Y\text{-}x)R_a \qquad I$$

wherein

x is a residue number selected from 31, 34, 35, 36, 37 or 38,

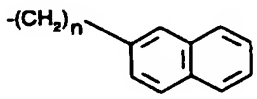
y is a residue number selected from 1, 2, 3, 4, 5, 6 or 7,

X^2 is Val or has independently one of the significances of X^{10},

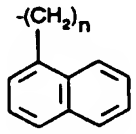
X^3 is Ser or has independently one of the significances of X^{10},

X^6 is Gln or has independently one of the significances of X^{10},

R^{10} is Asp or X^{10}, X^{10} being Trp or -NH-CHR'-CO- wherein R' is a radical of formula (a), (b), (c) or (d)



(a)



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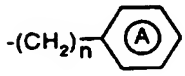

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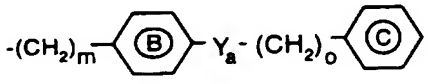


(d)

wherein

n is 1 or 2,

m is 1 or 2,

o is 0 or 1,

ring A is optionally substituted by one or more substituents

selected from fluoro, chloro, nitro, C_{1-4}alkyl and C_{1-4}alkoxy, whereby two alkyl or two alkoxy substituents may also form together a ring structure fused to ring A,
each of rings B and C independently may be substituted as indicated above for ring A, and
Y_a is a direct bond, $-CH_2-$, O, NH or N-C_{1-6}alkyl,
D is an amino acid sequence derived from an N-terminal fragment of PTHrP or PTH,
each of p, q and s is 1, provided that p is 0 when y > 2, q is 0 when y > 3 and s is 0 when y > 6,
R is H, R"-CO-, R"-O-CO-, R"-O-CH_2-CO-, R"-SO_2-, R''', R'''-NH-CO-, R'''-NH-CS- or NH_2-C_{1-6}alkylene-CO- wherein R" is C_{1-8}alkyl; ω-carboxy-C_{1-6}alkyl; ω-[(C_{1-4}alkoxy)-carbonyl]-C_{1-6}alkyl; C_{2-8}alkenyl; C_{5-7}cycloalkyl; C_{5-7}cycloalkyl-C_{1-4}alkyl; or phenyl, phenyl-C_{1-4}alkyl, 1-naphthyl, 2-naphthyl, 1-naphthyl-C_{1-2}alkyl or 2-naphthyl-C_{1-2}alkyl each of which being optionally ring substituted by one or more substitutents selected from fluoro, chloro, nitro, C_{1-4}alkyl and C_{1-4}alkoxy; heteroaryl; and
R''' has indepedently one of the significances given for R" except the significances of ω-carboxy-C_{1-6}alkyl and ω-[(C_{1-4}alkoxy)-carbonyl]-C_{1-6}alkyl; and
R_a is OH or NH_2,
with the proviso that at least one of X^2 and R^{10} has the significance of X^{10}.

When the substitutents of ring A, B or C form together a ring structure, it may be e.g. $-O-CH_2-CH_2-O-$. Example of polycyclic cycloalkyl is e.g. adamantyl.

By heteroaryl as R" is meant a 5-, 6- or 7-membered unsaturated heterocyclic radical comprising at least one nitrogen atom and optionally further a heteroatom such as N, O or S, and optionally condensed with a benzene ring. Heteroaryl is preferably indolyl, quinolyl or isoquinolyl.

The compounds of the invention may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and the acetates. Complexes are e.g. formed from the PTHrP or PTH compound of the invention on addition of inorganic

substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or an addition of polymeric organic substances.

In the compounds of formula I, the following significances are preferred either individually or in any combination or sub-combination:

1. D is an N-terminal fragment of hPTHrP.

2. D is an N-terminal fragment of hPTH.

3. X^{10} is Trp.

4. X^{10} is -NH-CHR'-CO- wherein R' is a radical of formula (a), (b) or (c), preferably (a) or (c), more preferably (a).

5. X^{2} is Trp or -NH-CHR'-CO- wherein R' is a radical of formula (a), (b) or (c),

6. Each of X^{2} and X^{10} is Trp or -NH-CHR'-CO- wherein R' is a radical of formula (a), (b) or (c).

7. Each of X^{3} and X^{10} is Trp or -NH-CHR'-CO- wherein R' is a radical of formula (a), (b) or (c).

8. Each of X^{6} and X^{10} is Trp or -NH-CHR'-CO- wherein R' is a radical of formula (a), (b) or (c).

9. Each of X^{2}, X^{6} and X^{10} is Trp or -NH-CHR'-CO- wherein R' is a radical of formula (a), (b) or (c).

10. n is 1.

11. y is a residue number selected from 3, 4, 5, 6 or 7, preferably 3 or 5.

12. x is a residue number selected from 31 or 34.

13. R is R"CO-, R"-SO_2, R''' or H_2N-C_{1-6}alkylene-CO-.

14. R" is C_{1-8}alkyl, phenylC_{1-4}alkyl, 1- or 2-naphthyl, 1- or 2-naphthyl-C_{1-2}alkyl.

15. R is ω-carboxy-C_{1-6}alkyl.

As already mentioned, one or more amino acid residues at positions other than 2 and/or 10 may be further replaced by a natural or unnatural amino acid residue as indicated above or be omitted. When the compounds of the invention are hPTHrP derivatives, they may comprise further amino acid replacement, e.g. in position 3, e.g. Ala, in position 4, e.g. Trp, in position 5, e.g. optionally ring-substituted Phe, in position 11 and/or 13, e.g. Leu, or in position 34, e.g. D-Ala; their amino acid sequence being optionally shortened at the N-terminus by the omission of 1 up to 7 amino acid residues. When the compounds of the invention are hPTH derivatives, amino acid residues may be further replaced e.g. in at least one of the positions selected from 8, 16, 18, 33 and 34, e.g. Leu^{8}, Ala^{16}, Gln^{18}, Thr^{33}, Ala^{34} or D-isomers thereof.

The present invention also provides a process for the production of the PTHrP or PTH compounds of the invention. They may be prepared in a stepwise manner either in solution or using the solid phase synthesis process or genetic engineering or by a combination of these methods.

The compounds of the invention may be produced for example as follows by:

a) removing at least one protecting group which is present in a PTHrP or PTH compound of the invention, e.g. a compound of formula I, in protected form; or

b) linking together by an amide bond two peptide fragments in protected, partially protected or unprotected form, the peptide fragments being such that the amino acid sequence of the desired PTHrP or PTH compound, e.g. of formula I, is obtained, and then effecting optionally stage a) of the process, or

c) adding a protecting group or substituent in a selective manner to the amino group of the N-terminal residue of the desired sequence or N-terminal fragment thereof in protected or unprotected form and then optionally carrying out step a),

and recovering the PTHrP or PTH compounds thus obtained in free form, in salt form or in complex form.

Process steps a), b) and c) may be effected in analogy with known methods, e.g. as known in the art of peptide chemistry or as described in the following examples. Where desired, in these reactions, protecting groups which are suitable for use in peptides may be used for functional groups which do not participate in the reaction. The term protecting group may also include a polymer resin having functional groups.

When the compounds of the invention comprise unnatural and natural residues, they may be produced by a combination of a chemical stepwise process and genetic engineering: the peptide sequence (whether the complete sequence or a fragment) made of genetically encodable amino acid residues may be produced by recombinant techniques and the desired unnatural amino acids or terminal amino substituent may be introduced by chemical synthesis and, where required, the fragments may be combined and the protecting group(s) when present may be removed.

In the following Examples all temperatures are in °C. The following abbreviations are employed.

DMF	=	dimethylformamide
DMA	=	N,N-dimethylacetamide-6-sulfonyl
Pmc	=	2,2,5,7,8-pentamethylchroman
tBu	=	tert.butyl
PyBOP	=	Benzotriazol-1-yl-oxy-tripyrrolidino-phosphonium hexafluorophosphate
Nal(2)	=	L-3-(2-naphthyl)-alanine
DIEA	=	N,N-diisopropyl-N-ethylamine
Fmoc	=	9-fluorenylmethoxycarbonyl
RT	=	room temperature
MS	=	M$^+$ determined by electrospray spectroscopy

EXAMPLE 1: [Trp^{4,10}]hPTHrP(1-34) OH

The peptide is synthesized in a stepwise manner on a polystyrene based resin support. The alpha-amino group is protected by Fmoc and the side-chain functional groups are protected as follows: Asp(OtBu), Glu(OtBu), His(Trt), Lys(Boc), Asn(Trt), Gln(Trt),

Arg(Pmc), Ser(tBu), Trp(Boc) and Thr(tBu).

Fmoc-Ala-oxymethyl-4-phenoxymethyl-co(polystyrene-1%-divinyl-benzene), approx. 0.5 mmol/g, is used as a starting material for the stepwise solid phase synthesis of peptides which consists of repetitive cycles of alpha-amino group deprotection, washing, coupling (i.e., attachment of next amino acid residue to the growing peptide chain) and washing. The N-alpha Fmoc group is cleaved using piperidine, 20% in DMA. In the coupling reaction four equivalents of Fmoc-amino acid and PyBOP-reagent and eight eqivalents of DIEA in DMA are used per amino-group. After complete assembly of the peptide chain the terminal Fmoc-protecting group is removed with piperidine in DMA as before. The peptide is cleaved from the resin support and all side-chain protecting groups are simultanously removed by using a reagent consisting of 5% of dodecylmethylsulfide and 5% water in TFA for two hours at RT. Resin particles are filtered off, washed with some TFA and the product is precipitated from the combined filtrates by the addition of 10 to 20 volumes of diethyl ether, washed with ether and dried. The product is purified by chromatography on a C-18 wide-pore silica column using a gradient of acetonitrile in 2% aqueous phosphoric acid. Fractions containing the pure compound are collected, filtered through an anion-exchange resin (Biorad, AG4-X4 acetate form) and lyophilized to give the title compound.

MS: 4146

In analogy with the procedure of Example 1, but using the appropriate amino-acids the following compounds may be prepared:

EXAMPLE 2: [Trp^{10}, Leu^{11}, Leu^{13}]hPTHrP(1-34)OH MS: 4059

EXAMPLE 3: [Trp^{10}, Leu^{11}]hPTHrP(1-34)OH MS: 4073

EXAMPLE 4: [$Trp^{3,10}$]hPTHrP(1-34)OH MS: 4188

EXAMPLE 5: [$Trp^{2,10}$]hPTHrP(1-34)OH MS: 4176

EXAMPLE 6: [Trp^{10}]hPTHrP(2-34)OH MS: 4018.3

EXAMPLE 7: [Trp^{10}]hPTHrP(3-34)OH MS: 3919.2

EXAMPLE 8: [Trp^{2}]hPTHrP(1-34)OH MS: 4104.9

EXAMPLE 9: [Trp^{10}]hPTH(1-34)OH MS: 4189

EXAMPLE 10: [Leu^{8},Trp^{10},Ala^{16},Gln^{18},Thr^{33},Ala^{34}]hPTH(1-34)OH MS: 4036

EXAMPLE 11: [Leu^{8},Trp^{10},Ala^{16},Gln^{18},Thr^{33},Ala^{34}]hPTH(2-34)OH
MS: 3949

EXAMPLE 12: [Leu^{8},Trp^{10},DLeu^{11},Gln^{18},Thr^{33},Ala^{34}]hPTH(1-34)OH
MS: 4079

EXAMPLE 13: [Phe^{2},Trp^{10}]hPTHrP(1-34)-OH MS: 4137.1
EXAMPLE 14: [Trp^{2},Trp^{10}]hPTHrP(2-34)-OH MS: 4105.1
EXAMPLE 15: [Trp^{6},Trp^{10}]hPTHrP(1-34)-OH MS: 4146.8
EXAMPLE 16: [Trp^{6},Trp^{10}]hPTHrP(6-34)-OH MS: 3623.3
EXAMPLE 17: [Trp^{10}]hPTHrP(7-34)-OH MS: 3437.6
EXAMPLE 18: [Trp^{3},Trp^{10}]hPTHrP(3-34)-OH MS: 4018.0
EXAMPLE 19: [Trp^{10}]hPTHrP(1-34)OH MS: 4088.7

EXAMPLE 20: [Trp^{6}, Trp^{10}]hPTHrP(3-34)NH_2 MS: 3976.8
EXAMPLE 21: [Phe^{6}, Trp^{10}]hPTHrP(1-34)OH MS: 3546.1
EXAMPLE 22: [Phe^{2}, Phe^{6}, Trp^{10}]hPTHrP(1-34)OH MS: 4155.1

EXAMPLE 23: N$^{\alpha}$-3-aminopropionyl-[Phe^{6}, Nal(2)^{10}]hPTHrP(4-34)NH_2
MS: 3932.0

EXAMPLE 24: N$^{\alpha}$-benzyloxycarbonyl[Nal(2)^{10}]hPTHrP(3-34)-NH_2

This peptide is prepared in a manner analogous to Example 1 but using 4-(2',4'-dimethoxyphenyl-Fmoc-amino-methyl)-phenoxy-co(polystyrene-1%-divinylbenzene), approx. 0.4 mmol/g, which may be prepared, e.g., as described in Tetrah. Letters, 28:3787-3790 (1987) as a starting material. In the last synthesis cycle Z-Ser-OH is added to the peptide chain and the peptide cleaved and purified as in Example 1.
MS: 3975.3

EXAMPLE 25: N$^{\alpha}$-acetyl-[Trp^{10}]hPTHrP(3-34)OH

This peptide is assembled in analogy with the procedure of Example 1. At the end of the synthesis a final cycle of Fmoc-deprotection and acetylation using a 1:1 mixture of acetic anhydride and DMF is applied. The peptide is cleaved from the resin and purified as before.
MS: 3961.1

The following compounds may be prepared in a manner analogous to that of Examples 1, 24 or 25.

EXAMPLE 26: N^{α}-acetyl-[Trp^{10}]hPTHrP(2-34)OH
MS: 4060.4

EXAMPLE 27: 1-Isocaproyl-[Leu^{8},Trp^{10},Gln^{18},Thr^{33},Ala^{34}]-hPTH(1-34)OH
MS: 4091

EXAMPLE 28: N^{α}-2-naphthyl-acetyl-[Nal(2)^{10}]hPTHrP(3-34)NH_2
MS: 4097.1

EXAMPLE 29: N^{α}-2-naphthyl-acetyl-[Nal(2)^{10}]hPTHrP(4-34)NH_2
MS: 4009.7

EXAMPLE 30: N^{α}-2-naphthyl-sulfonyl-[Nal(2)^{10}]hPTHrP(3-34)NH_2
MS: 4119.2

EXAMPLE 31: N^{α}-2-naphthoxyacetyl-[Nal(2)^{10}]hPTHrP(3-34)NH_2
MS: 4111.5

EXAMPLE 32: N^{α}-2-naphthylacetyl-[Ala^{3},Nal(2)^{10}]hPTHrP(3-34)NH_2
MS: 4080.8

EXAMPLE 33: N^{α}-benzyloxycarbonyl-[Trp^{2},Nal(2)^{10}]hPTHrP(2-34)NH_2
MS: 4248.3

EXAMPLE 34: 3-Naphth-2-yl-proprionyl-[Nal(2)^{10}]hPTHrP(3-34)NH_2
MS: 4110.8

EXAMPLE 35: 3-Naphth-2-yl-propionyl-[Nal(2)^{10}]hPTHrP(3-34)NH_2
MS: 4110.9

EXAMPLE 36: N^{α}-2-naphthyl-acetyl-[Nal(2)^{10},DAla^{34}]hPTHrP(3-34)NH_2
MS: 4095.9

EXAMPLE 37: N^{α}-2-naphthyl-acetyl-[Nal(2)^{10}]hPTHrP(3-31)NH_2
MS: 3788.0

EXAMPLE 38: N^{α}-3-naphth-2-yl-propionyl-[Nal(2)^{10}]hPTHrP(7-34)NH_2
MS: 3630.0

EXAMPLE 39: N^{α}-acetyl-[Phe^{6},Nal(2)^{10}]hPTHrP(6-34)NH_2
MS: 3637.0

EXAMPLE 40: N^{α}-acetyl-[Phe^{6},Nal(2)^{10}]hPTHrP(4-34)NH_2
MS: 3903.1

EXAMPLE 41: 1-(1-amino-1-cyclopentyl-carbonyl)-[Leu^{8},Trp^{10},Gln^{18},Thr^{33},Ala^{34}]hPTH(1-34)OH
MS: 4104

Fmoc-1-aminocyclopentane-1-carboxylic acid used in the preparation of the peptide resin intermediate may be prepared, e.g. as described by G. Valle et al.,1988, in Can.J.Chem.66:2575-2582.

EXAMPLE 42: 1-Adamantyl-carbonyl-[Leu^{8},Trp^{10},Gln^{18},Thr^{33},Ala^{34}]hPTH(1-34)OH
MS: 4155

EXAMPLE 43: 1-(3-indolyl-carbonyl)-[Leu^{8},Trp^{10},Gln^{18},Thr^{33},Ala^{34}]hPTH(1-34)OH
MS: 4150

EXAMPLE 44: 1-(3-quinolyl-carbonyl-,[Leu^{8},Trp^{10},Ala^{16},Gln^{18},Thr^{33},Ala^{34}]hPTH(1-34)OH
MS: 4104

EXAMPLE 45: 1-(2-naphthoyl)-[Leu^{8},Trp^{10},Gln^{18},Thr^{33},Ala^{34}]-hPTH(1-34)OH
MS: 4147

Example 46: N^{α}-(2-naphthyl)-methyl-[Trp^{10}]hPTHrP(2-34)-OH

Methyl N-naphthoyl(2)-valinate is treated with Lawesson's reagent, S-O. Lawesson et al., Tetrahedron 37:3635 (1981) and the resulting methyl N-thionaphthoyl(2)-valinate is reduced using the procedure described for endothiopeptides by F.S. Guziec et al., Tetrah. Letters 23-26 (1990). The methyl ester is hydrolyzed using LiOH and N-alpha-(naphtyl(2)-methyl)-valine hydrochloride obtained as a crystalline solid, mp = 205-210° (dec.). This is coupled using PyBOP-reagent to previously prepared, protected [Trp^{10}]hPTHrP-(3-34)-O-peptide resin from which the N-alpha Fmoc-group has been removed. The peptide is cleaved from the resin and

purified as in Example 1.

MS: 4158.2

EXAMPLE 47: N^{α}-2-naphth-2-yl-ethyl-[Trp^{10}]hPTHrP(2-34)OH
MS: 4171.9

EXAMPLE 48: N^{α}-2-naphth-2-yl-ethyl-[Ala^{3},$Nal(2)^{10}$]hPTHrP(3-34)NH_2
MS: 4066.7

EXAMPLE 49: N^{α}-succinyl-[Phe^{6},$Nal(2)^{10}$]hPTHrP(5-34)NH_2

This peptide is prepared in a manner analogous to Example 1 but using 4-(2',4'-dimethoxyphenyl-Fmoc-amino-methyl)-phenoxy-co(polystyrene-1%-divinylbenzene), 0.63g, loading approx. 0.4 mmol/g, which may be prepared, e.g., as described in Tetrah. Letters, 28: 3787-3790 (1987) as a starting material. After coupling the last amino acid, Fmoc-His(Trt)-OH, the Fmoc-group is selectively removed as usual and the peptide resin reacted with succinic anhydride (0.5g) and DIPEA (0.86ml) in DMF. The peptide is cleaved from the resin, purified and lyophilized in the acetate form as in Example 1 to give the title compound.

MS: 3832.0

By following the procedure of Example 49, following compounds may be prepared:

EXAMPLE 50: N^{α}-succinyl-[Phe^{6},$Nal(2)^{10}$]hPTHrP(5-31)NH_2
MS: 3522.1

EXAMPLE 51: N^{α}-succinyl-[$Nal(2)^{6}$,$Nal(2)^{10}$]hPTHrP(5-34)NH_2
MS: 3881.7

EXAMPLE 52: N^{α}-glutaryl-[Phe^{6},$Nal(2)^{10}$]hPTHrP(5-34)NH_2
MS: 3845.6

EXAMPLE 53: N^{α}-succinyl-[Phe^{6},$Nal(2)^{10}$,$DAla^{34}$]hPTHrP(5-34)NH_2
MS: 3832.0

EXAMPLE 54: N^{α}-succinyl-[4-Cl-Phe^{6},$Nal(2)^{10}$]hPTHrP(5-34)NH_2
MS: 3866.5

EXAMPLE 55: N^{α}-succinyl-[4-Cl-Phe^5, 4-Cl-Phe^6, $Nal(2)^{10}$]-hPTHrP(5-34)NH_2

MS: 3910.2

The compounds of the invention in free form or in the form of pharmaceutically acceptable salts and complexes exhibit valuable pharmacological properties as indicated in animal tests and are therefore indicated for therapy.

The biological activity of the compounds of the invention is assessed in vitro by measuring their ability of stimulating (agonism) or inhibiting the PTH-stimulated (antagonism) synthesis of cyclic AMP in UMR 106-06 rat osteosarcoma cells according to the method of Marcus and Aurbach in Endocrinology, 85, 801-810 (1969). Rat osteosarcoma UMR 106 cells are grown to confluence in DMEM-HAM's F12 medium (1:1) - 10% FCS in 12 well plates. The medium is then changed to medium with 2% FCS and 1-5 µCi/well [3H]-adenine is added. Two hours later, cells are washed twice with serum-free medium and incubated in DMEM - 0,1% BSA containing 1 mM 3-isobutyl-1-methylxanthine to exclude actions on phospho- diesterases. Test substances are added 20 min later either alone or together with PTH (antagonist experiment). After 15 min, the medium is removed and the reaction is stopped and cAMP extracted by adding 0.5 ml ice cold 5% trichloroacetic acid. A carrier solution (0.5 ml/well) containing 0.2 mM of unlabelled adenine, adenosine, AMP, ADP, ATP, and cAMP as well as 0.4 µCi [14C]-adenosine for determination of recovery is added. [3H]-cAMP is separated using serial Dowex AG 50W-X4 (200-400 mesh) and alumina chromatography and counted according to Salomon Y. in Advances in Cyclic Nucleotide Research, Vol. 10, Raven Press, 35-55, 1979. Results are calculated in % of solvent control and EC_{50} values determined from DRC curves. Antagonist potency is calculated from the right ward shift of DRC curves of PTHrP or PTH and is given as pA_2 values. Compounds of the invention are active as antagonists at a concentration of 10^{-9} to 10^{-5} M. Compound of Examples 36, 37 and 49 have a pA_2 value in the UMR 106-06 cells of 10.3; 9.7 and 9.3, respectively.

The compounds of the invention also have binding affinity to PTH receptors, e.g. as follows:

Chicken [Tyr^{36}]PTHrP(1-36)amide is iodinated to a specific activity of 2,200 Ci/mmol using the lactoperoxidase method (Anawa Lab. AG, Wangen). Monolayers of opossum kidney cells (OK1) are washed with 200 µl DMEM and HAM's F12 (1:1) containing 1% BSA and incubated at 16°C with 50.000cpm of [^{125}I-Tyr^{36}]chPTHrP(1-36)amide per well in the presence or absence of 1 µM [Tyr^{36}]chPTHrP-(1-36)amide. After incubation, cells are washed with 0.5 ml medium (4°C), lysed with 0.5 ml 1N NaOH and radioactivity is determined. Specific binding is defined as total binding minus nonspecific binding. Competition curves are analyzed using SCTFIT, a non-linear regression computer program (Feyen et al, 1992, Biochem. Biophys. Res. Commun. 187:8-13) and data presented as mean pK_D values (n=2 to 3). Compounds of Examples 36, 37 and 49 have a pK_D value of 8.3; 7.9 and 8.4, respectively.

Furthermore, the compounds of the invention antagonize the effect of PTH after i.v. infusion, e.g. as determined in thyroparathyroidectomized rats. 24 h after thyroparathyroidectomy, anesthetized rats are infused with PTH(1-34) and the compound to be tested via separate jugular veins. Urine is collected from the urinary bladder which is cannulated via the ventral approach. Phosphate and cAMP content in the urine and calcium and phosphate in serum are measured using standard methodology. These parameters are used to quantify antagonist potencies against PTH effects in vivo. In this test, the compounds of the invention antagonize the PTH effects when administered by i.v. infusion at a dose of from 1 µg/kg/h to 1 mg/kg/h. Compound of Example 49 completely suppresses PTH-induced phosphaturia for up to 90 min when i.v. infused at 190 µg/kg/h, PTH(1-34) being i.v. infused at 4 µg/kg/h.

The compounds of the invention are accordingly useful for preventing or treating all conditions which are associated with increased plasma calcium caused by excessive release of PTH or PTHrP e.g. hyperparathyroidism, hypercalcemia, e.g. associated with malignancies, e.g. breast carcinomas, squamous cell carcinomas of the lung, esophagus and head and neck region and hematological malignancies, with or without bone metastases. The compounds of the invention are furthermore useful for the prevention or treatment of tumour growth, tumour penetration and ingrowth in bones stimulated by PTHrP, for treating dermatological disorders, e.g. tissue repair therapies, for

example treatment of burns, ulcerations and wounds, and for hair growth promotion.

For these indications, the appropriate dosage will, of course, vary depending upon, for example, the host, the mode of administration and the severity of the conditions being treated. However, in general, satisfactory results in animals are indicated to be obtained at daily dosages from about 0.1 to about 100 mg/kg animal body weight. In larger mammals, for example humans, an indicated daily dosage is in the range from about 0.1 to about 500 mg of the compounds of the invention.

The compounds of the invention may be administered in free form or in pharmaceutically acceptable salt form or complexes. Such salts and complexes may be prepared in conventional manner and exhibit the same order of activity as the free compounds. The present invention also provides a pharmaceutical composition comprising a compound of the invention in free base form or in pharmaceutically acceptable salt form or complex form in association with a pharmaceutically acceptable diluent or carrier. Such compositions may be formulated in conventional manner. Unit dosage forms suitably comprise from about 0.025 to 250 mg of a compound of the invention, together with a pharmaceutical acceptable diluent or carrier therefor.

The compounds of the invention may be administered by any conventional route, for example parenterally e.g. in form of injectable solutions or suspensions, or in a nasal or a suppository form. The compounds of the invention may alternatively be administered e.g. topically in the form of a cream, gel or the like for example for the treatment of the skin or hair growth as hereinbefore described.

In accordance with the foregoing the present invention further provides:

a) a compound of the invention or a pharmaceutically acceptable salt or complex thereof for use as a pharmaceutical;

b) a method for preventing or treating conditions and disorders as indicated above in a subject in need of such treatment, which method comprises administering to said subject an

effective amount of a compound of the invention or a pharmaceutically acceptable salt or complex thereof;

c) a compound of the invention or a pharmaceutically acceptable salt or complex thereof for use in the preparation of a pharmaceutical composition for use in the method as in b) above.

According to a further embodiment of the invention, the compounds of the invention may be employed as adjunct or adjuvant to other therapy, e.g. in hypercalcemia to a therapy using a bone resorption inhibitor, in particular a therapy employing a calcitonin or an analogue or derivative thereof, e.g. salmon, eel or human calcitonin, a biphosphonate, a diuretic or any combination thereof, or in case of tumour therapy, a cytostatic agent or any combination thereof.

In accordance with the foregoing the present invention provides in a yet further aspect:

d) a method for preventing or treating hypercalcemia for example for preventing or treating any of the specific conditions or diseases hereinbefore set forth, in a subject in need of such a treatment which method comprises administering to said subject an effective amount of a) a compound of the invention and b) a second drug substance, said second drug substance being a therapeutic agent as indicated above.

Compounds of Examples 36, 37 and 49 are preferred for preventing or treating all conditions which are associated with increase plasma calcium caused by excessive release of PTH or PTHrP.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: SANDOZ LTD.
(B) STREET: Lichtstrasse 35,
(C) CITY: BASLE
(E) COUNTRY: SWITZERLAND
(F) POSTAL CODE (ZIP): CH-4002

(A) NAME: SANDOZ PATENT GMBH
(B) STREET: Humboldtstrasse 3
(C) CITY: LOERRACH
(E) COUNTRY: GERMANY
(F) POSTAL CODE (ZIP): D-79539

(A) NAME: SANDOZ ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H.
(B) STREET: Brunner Strasse 59
(C) CITY: VIENNA
(E) COUNTRY: AUSTRIA
(F) POSTAL CODE (ZIP): A-1230

(ii) TITLE OF INVENTION: PEPTIDES

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ala Val Ser Glu His Gln Leu Leu His Asn Lys Gly Lys Ser Ile Gln
1 5 10 15

Asn Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu Ile Arg Ala
35

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ala Val Ser Glu His Gln Leu Leu His Asn Lys Gly Lys Ser Ile Gln
1 5 10 15

Asn Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ala Val Ser Glu His Gln Leu Leu His Asn Lys Gly Lys Ser Ile Gln
1 5 10 15

Asn Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile
20 25 30

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ala Leu Gly
35

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val
20 25 30

CLAIMS

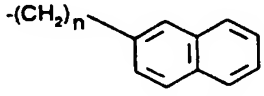
1. A PTH or PTHrP compound in which at least one of the amino acid residues naturally occurring in positions 2 and 10 is replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain, and optionally at least one of the amino acid residues naturally occurring in positions 3 and 6 is further replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain,

in free form or in salt or complex form.

2. A PTH or PTHrP compound in which the amino acid residue naturally occurring in position 10 is replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain, and optionally at least one of the amino acid residues naturally occurring in positions 3 and 6 is further replaced by tryptophane or another amino acid residue bearing an aromatic or heteroaromatic group on its side chain,

in free form or in salt or complex form.

3. A compound according to claim 1 wherein the amino acid residue bearing an aromatic or heteroaromatic group on its side chain is an amino acid residue wherein the side chain is optionally ring-substituted 3- or 4-pyridyl-methyl, 3-indolyl-methyl or 3-indazolyl-methyl or a radical of formula (a), (b), (c) or (d)



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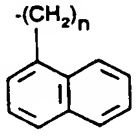

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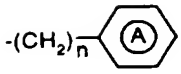

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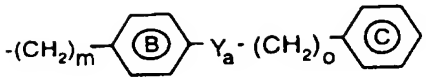


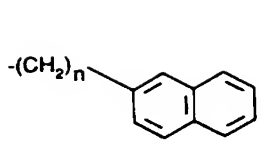
(d)

wherein
n is 1 or 2,
m is 1 or 2,
o is 0 or 1,
ring A is optionally substituted by one or more substituents selected from fluoro, chloro, nitro, C_{1-4}alkyl and C_{1-4}alkoxy, whereby two alkyl or two alkoxy substituents may also form together a ring structure fused to ring A,
each of rings B and C independently may be substituted as indicated above for ring A, and
Y_a is a direct bond, $-CH_2-$, O, NH or $N-C_{1-6}$alkyl.

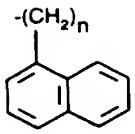
4. A compound according to claim 1, which is a compound of formula I

$$R-[(X^2)_p,(X^3)_q,(X^6)_s,R^{10}]-D-(y-x)R_a \qquad I$$

wherein
x is a residue number selected from 31, 34, 35, 36, 37 or 38,
y is a residue number selected from 1, 2, 3, 4, 5, 6 or 7,
X^2 is Val or has independently one of the significances of X^{10},
X^3 is Ser or has independently one of the significances of X^{10},
X^6 is Gln or has independently one of the significances of X^{10},
R^{10} is Asp or X^{10}, X^{10} being Trp or -NH-CHR'-CO- wherein R' is a radical of formula (a), (b), (c) or (d)



(a)



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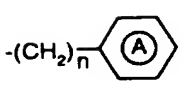

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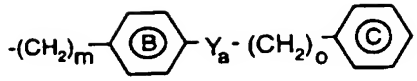

(d)

wherein
n is 1 or 2,
m is 1 or 2,
o is 0 or 1,
ring A is optionally substituted by one or more substituents selected from fluoro, chloro, nitro, C_{1-4}alkyl and C_{1-4}alkoxy, whereby two alkyl or two alkoxy substituents may also form together a ring structure fused to ring A, each of rings B and C independently may be substituted as indicated above for ring A, and
Y_a is a direct bond, $-CH_2-$, O, NH or N-C_{1-6}alkyl,
D is an amino acid sequence derived either from an N-terminal fragment of PTHrP or PTH,
each of p, q and s is 1, provided that p is 0 when y > 2, q is 0 when y > 3 and s is 0 when y > 6,
R is H, R"-CO-, R"-O-CO-, R"-O-CH_2-CO-, R"-SO_2-, R''', R'''-NH-CO-, R'''-NH-CS or NH_2-C_{1-6}alkylene-CO- wherein R" is C_{1-8}alkyl; ω-carboxy-C_{1-6}alkyl; ω-[(C_{1-4}alkoxy)-carbonyl]-C_{1-6}alkyl; C_{2-8}alkenyl; C_{5-7}cycloalkyl; C_{5-7}cycloalkyl- C_{1-4}alkyl; or phenyl, phenyl-C_{1-4}alkyl, 1-naphthyl, 2-naphthyl, 1-naphthyl-C_{1-2}alkyl or 2-naphthyl-C_{1-2}alkyl each of which being optionally ring substituted by one or more substitutents selected from fluoro, chloro, nitro, C_{1-4}alkyl and C_{1-4}alkoxy; heteroaryl; and
R''' has indepedently one of the significances given for R" except the significances of ω-carboxy-C_{1-6}alkyl and ω-[(C_{1-4}alkoxy)-carbonyl]-C_{1-6}alkyl; and

R_a is OH or NH_2,
with the proviso that at least one of X^2 and R^{10} has the significance of X^{10}.

5. A compound according to claim 1 or 4 which is derived from the N-terminal fragment of hPTH or hPTHrP.

6. A compound according to claim 1 which is selected from
N^{α}-2-naphthyl-acetyl-[Nal(2)^{10},DAla^{34}]hPTHrP(3-34)NH_2
N^{α}-2-naphthyl-acetyl-[Nal(2)^{10}]hPTHrP(3-31)NH_2
N^{α}-succinyl-[Phe^{6},Nal(2)^{10}]hPTHrP(5-34)NH_2

7. A process for the production of a compound according to claim 1, in free form or in salt or complex form, which process comprises

a) removing at least one protecting group which is present in a PTHrP or PTH compound of the invention, e.g. a compound of formula I, in protected form; or

b) linking together by an amide bond two peptide fragments in protected, partially protected or unprotected form, the peptide fragments being such that the amino acid sequence of the desired PTHrP or PTH compound, e.g. of formula I, is obtained, and then effecting optionally stage a) of the process, or

c) adding a protecting group or substituent in a selective manner to the amino group of the N-terminal residue of the desired sequence or N-terminal fragment thereof in protected or unprotected form and then optionally carrying out step a),

and recovering the PTHrP or PTH compounds thus obtained in free form, in salt form or in complex form.

8. A compound according to claim 1 in free form or in physiologically acceptable salt form for use as a pharmaceutical.

9. A pharmaceutical composition comprising a compound according to claim 1, in free form or in physiologically acceptable salt form, together with a pharmaceutically acceptable

diluent or carrier therefor.

10. A compound according to claim 1 in free form or in physiologically acceptable salt form for use as a pharmaceutical, in association with a further therapeutic agent selected from a bone resorption inhibitor and a cytostatic agent.

11. A method for preventing or treating conditions which are associated with increased plasma calcium caused by excessive release of PTH or PTHrP, for preventing or treating tumor growth stimulated by PTHrP, for treating dermatological disorders and for hair growth promotion, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound according to claim 1 in free form or in a physiologically acceptable salt form.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/02993

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/635 A61K38/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 269 176 (SANDOZ LTD) 2 February 1994 see page 1 - page 30; examples 82,302; claims 1,2,5,6,8-10,14,16-22,24-27,44-46 ---	1-5,7-10
A	WO,A,92 00753 (UNIV CALIFORNIA) 23 January 1992 see the whole document ---	1-11
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 3, 25 January 1991 MD US, pages 1997-2004, F.E.COHEN ET AL 'Analogues of PTH modified at positions 3 and 6' see the whole document --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 November 1995	01.12.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/02993

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 451 867 (MITSUBISHI CHEM IND) 16 October 1991 see the whole document -----	1-11

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

I ...national application No.
PCT/EP 95/02993

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 11
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 11 is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/02993

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A-2269176	02-02-94	AU-B-	4156693	20-01-94
		CA-A-	2100423	16-01-94
		CN-A-	1099801	08-03-95
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		US-A-	5229489	20-07-93